In addition to those described here, other groups were treated in a way similar to that for the EUB, EUC, NB, and NC groups described above. The uranium used for the first two groups was 85% enriched. A number of mice from each group were sacrificed at various time intervals following treatment to obtain a more complete series of tissue samples for histological study. Blood counts were done routinely on mice from each of these groups. The results obtained in these studies will be presented in detail elsewhere. Briefly, they amplified and confirmed the results reported above. One day after bombardment both NB and EUB groups developed leucopenia. After the third day, the leucocyte count of group NB began to increase, whereas that of group EUB continued to drop. The white blood cell count of EUC mice remained normal for several days and then decreased slightly. Three or four days after bombardment, anemia began to develop in mice of group EUB and continued to increase in severity. Anemia also developed in EUC, but to a less marked degree. No change occurred in the erythrocyte count of NB animals. The spleen size of group EUB animals started to decrease immediately after bombardment and continued to drop until the weight reached $\frac{1}{8}$ of the original; NB spleens decreased in weight and recovered after 3–5 days; EUC spleens slowly decreased in size because of the alpha activity of the uranium. On the first day after bombardment microscopic examination of the liver showed central hydropic degeneration with swollen pale cells in the lobules. In certain areas there was frank necrosis with loss of cell outline and destruction of nuclei and scattered nuclear debris. In animals examined two days later these findings were much less evident, the livers having more normal histological appearance. In one case many mitotic figures were found scattered throughout the sections. Still later, the livers appeared normal. These findings correspond to the histological picture one obtains in the liver after a massive dose of whole body X-rays (*14*). In the EUC group, about half of the liver sections examined exhibited the same sort of central degeneration of hepatic cells as was found in the EUB group; in the rest of the animals the livers appeared normal. Animals sacrificed in subsequent days in this group all had normal livers. The livers in the NC and NB groups were all entirely normal histologically.

A rough comparison can be made of the dose necessary to kill mice by fission and by beta particles from P^{32} in chromic phosphate. Acute lethal effects have been observed by Jones, *et al.* (*5*) when 100,000 r.e.p. was given by *in vivo* chromic phosphate to the mouse liver. The ratio of the phosphorus liver dose to the fission liver dose is 27; this value cannot be taken as final, but suggests that fission recoils are more effective in producing lethal effects than beta rays.

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Protection Against Bacterial Endotoxins by Penicillin and Its Impurities[1]

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Previous reports have described protection of mice against the endotoxins of meningococcus (*1*) and gonococcus (*6*) by the subcutaneous administration of large doses of penicillin. Further investigation demonstrated that such protection is best provided by repeated intraperitoneal injection of penicillin during the 24-hr period before endotoxin is given (*2*). Recent work has shown that mice can be protected by this same means against the endotoxins of a number of other gram-negative bacteria—*S. paratyphi* A, *S. paratyphi* B, *Shigella paradysenteriae, S. enteritidis, S. aertrycke,* and *A. aerogenes.* Negative results were obtained only with the endotoxin of *Shigella shigae.* It has also been found that certain impure penicillin preparations provide a much greater degree of protection than does crystalline penicillin.

Endotoxin is prepared by the method previously described (*1*) and is injected intraperitoneally in 1-cc volume. Protection is measured by comparing the LD_{50} of endotoxin (as computed by the Reed-Muench formula) for untreated and for penicillin-treated mice. A typical determination is presented in the experiment shown in Table 1.

It will be seen that the LD_{50} of endotoxin for mice treated with crystalline penicillin was 3 times that for

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untreated mice, and that the LD_{50} for mice treated with impure penicillin was 9 times as great. This finding has been confirmed by repeated experiments of the same kind using a single batch of endotoxin. Results are shown in Table 2.

TABLE 1

PROTECTIVE ACTION OF PENICILLIN AGAINST BACTERIAL ENDOTOXIN

Dose of endotoxin (*S. aertrycke*) (ml)	Numbers of mice killed by each dose of endotoxin		
	Saline control	Crystalline penicillin (mostly G)	Impure penicillin
.8	—	—	10/10
.4	—	—	6/10
.2	—	9/10	5/10
.1	9/10	5/10	0/10
.05	7/10	5/10	—
.025	5/10	2/10	—
.0125	3/10	—	—
LD_{50}*	.025 ml	.082 ml	.235 ml

* Computed by the Reed-Muench formula.

From these figures it is apparent that in a large series of experiments the LD_{50} of endotoxin for mice treated with impure penicillin is 5 times as great as that for control mice and more than twice as great as the LD_{50} for mice treated with crystalline penicillin.

TABLE 2

AVERAGE LD_{50} OF ENDOTOXIN (*S. aertrycke*) IN TREATED AND UNTREATED MICE

Control mice* (untreated)	Mice treated with:	
	crystalline penicillin	impure penicillin
.05 ml	.11 ml	.26 ml
Averages computed from:		
33 experiments 1,113 mice	9 experiments 300 mice	43 experiments 1,436 mice

* This control group includes mice receiving no injections before endotoxin, as well as mice receiving, instead of penicillin, injections of various control materials, e.g. physiological saline solution, corn-steep liquor, aleuronat suspension, etc.

That this protection is not due to the control of intercurrent infections has been demonstrated by routine autopsy cultures on a representative number of mice dying after injection of endotoxin.

The total dose of penicillin used in these experiments was 15,000 units/mouse. Greater amounts provided no additional protection. The penicillin was given in 3 doses at 20, 18, and 2 hrs before injection of endotoxin. Treatment after endotoxin or with a single large dose of penicillin seems to afford less protection.

The impure penicillin preparation[2] which we employ loses its protective activity when treated with sufficient heat or penicillinase to inactivate its penicillin, but its protective activity can be completely restored by the addition of crystalline penicillin in the amount originally present. Other investigators (*3, 4, 5, 7, 8, 9*) have recently reported that certain impurities associated with penicillin enhance its activity in the control of infections. We have not yet determined whether those are the same as the heat-stable impurity factor which we have herein described.

Addendum: Since the completion of the experiments recorded in Table 2, 28 more experiments have been performed using the same impure penicillin with certain improvements designed to reduce its toxicity and to increase its protective activity. The average LD_{50} of endotoxin for the entire group of animals treated with impure penicillin has thereby been raised to about 8 times the LD_{50} for the control animals.

Statistical analysis, using the method of the standard error, shows that a difference of this magnitude between the means of these two sets of data is likely to occur by chance less than one time in a million.

[2] An intermediate fraction in the purification of penicillin, kindly supplied by the Abbott Laboratories.

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The Folic Acid Activity and Antagonism of Two Structurally Related Derivatives of Benzimidazole[1]

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Folic acid is an essential growth factor for *Streptococcus faecalis* R. and other lactic acid bacteria. However, these microorganisms are known to develop without folic acid provided certain pyrimidine and purine bases are present. Thymine can be considered to replace folic acid in the nutrition of *Str. faecalis,* though much higher concentrations are required (*8*). Spies has shown that large doses of thymine elicit a hematopoietic response

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